Public Forum Sessions
(Morning and Afternoon)

Questions and Answers

Clinical Proteomic Technology Assessment for Cancer
Pre-Application Meeting
RFA-CA-07-012

Natcher Conference Center
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Office of the Director
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Public Forum: Questions and Answers (Excerpted from Meeting Report)

MORNING SESSION

Comment: It was suggested that biomarkers with supporting clinical data be prioritized for analysis and that biomarker analysis criteria be standardized. It also was suggested that a systematic scheme to prioritize focus (e.g., inflammatory mediators that have initial clinical data) would maximize the usefulness of data generated.

Question 1: *Can you clarify why this project emphasizes sequencing, when the project is not centered around biomarker discovery?*
Answer 1: Although discovery is not the primary theme of this project, the NCI does not intend to discourage discovery. The major goal of this project is the enhancement of the technological capabilities, standard protocols, and reproducibility of measurements across different laboratories and platforms. The NCI views this project as an initiative to enhance the capabilities for discovery, and the Institute recognizes that new features representative of cancer developmental processes will evolve from the supported projects. The Institute envisions the data that emerge from this initiative as tools to advance new candidates.

Q2: *Is the project goal to repeat previous efforts in de novo biomarker discovery or to examine the biology and identify proteins implicated in cancer pathways? Is searching for these proteins in the biofluid a viable strategy for this project?*
A2: Yes. An applicant may propose such a strategy, although it is not the only viable strategy. One objective of this project is to identify and reproducibly measure target proteins and peptides. While the Institute endorses the candidate-based approach, it is aware that a given strategy may propose the identification of certain proteins that are different from those ultimately identified in experimental analysis. Nonetheless, it is critical that the experimental design be conducted in such a way that two discrete sites may reproduce each other's data. Where possible, the NCI encourages groups to develop microsimulation models, since certain technologies tend to favor the identification of certain proteins and peptides. Using these models to identify targets likely to be found in serum or plasma is one approach that can be taken, but this project focuses more on identifying the variables that cause different laboratories to identify different proteins within the same sample set.

Q3: *What is meant by the term "peptide" in the context of this project?*
A3: "Peptide" in this context refers to a protein fragment. Identification of peptide fragments is one part of this initiative.

Q4: *Will Clinical Proteomic Technology Assessment for Cancer (CPTAC) consortia be expected to work with tissues other than biofluids such as plasma and serum?*
A4: Whenever available, each plasma or serum sample should be accompanied by the matching associated tumor tissue. In cases where tissue is used, appropriate protocols for biospecimen handling should be coordinated with the Office of Biorepositories and Biospecimen Research (OBBR). The NCI recognizes, however, that the tumor tissue may not be surgically removed in some instances. The Institute also recognizes that tissues and tissue-derived fluids are sources of biomarker discovery, and a tissue-based approach to standardization of protein or peptide detection is within the scope of the RFA. To be consistent with the requirements of the RFA, however, tissues and biological fluids must be made available for comparative analysis between platforms and laboratories. Therefore, applicants that wish to base their experimental designs on tissues or tissue-based fluids should be aware that the availability of those samples for replicate analyses on multiple platforms is an important consideration.

Q5: *Will CPTAC consortia investigators be expected to provide samples to investigators from the R21/R33-funded projects?*
A5: No. Although this practice is encouraged, it is not required. If such studies are feasible, however, the NCI will work with consortium members to facilitate interactions between investigators.

Q6: *Are there provisions for central data storage, or is data storage the responsibility of CPTAC members?*
A6: Currently, the NCI does not have plans to store data centrally for this project. Participating entities that generate data will maintain these data locally and utilize caBIG™ to allow others to access these data. At present, there are no plans for caBIG™ to supply storage space for data generated by awardees. [Note: This answer was provided by Dr. Komatsoulis in the afternoon session.]

Q7: *Does the NCI provide any guidelines with regard to sample pooling?*
A7: No. Sample pooling is acceptable as part of a proposed experimental design, although respondents should discuss the pros and cons of their proposed strategies. The NCI makes no value judgment for or against the practice of sample pooling; experimental designs that involve pooling should be supported with a rationale and a plan to address any relevant issues.

Q8: *What is the total sample volume or total amount of tumor tissue necessary to fulfill the requirements of the RFA in terms of sample sharing?*
A8: The quantity of sample necessary for this project depends on the experimental design. This RFA allows for the consideration of retrospective collections, and applicants that wish to include retrospective collections in their proposals should provide the volumes of those collections that will be available. For prospective collections, limitations on frequency of collection and other restrictions on use should be identified in the application. Determination of the sample volumes necessary to conduct analyses on certain platforms should be made by the management team responsible for interlaboratory studies. Applicants are encouraged to identify the upper and lower limits of sample volumes available from retrospective samples, the strategies for sample collection, and the capabilities in prospective collections.

Q9: *What is the sample collection timeframe for prospective studies that have just begun?*
A9: The NCI would like to see prospective collections completed during the second and third years of the program. The NCI does not expect prospectively collected samples to be available within the first year of the program. Applicants are encouraged, however, to map timeframes from companion studies into their experimental designs when possible.

Q10: *For this project, is the NCI interested in relative or absolute quantitation of proteins and peptides?*
A10: Both approaches are applicable to this project.

Q11: *Will the NCI assist grantees in obtaining relatively inexpensive, high-quality peptide standards that can be used in quantitation experiments?*
A11: Yes. The NCI is currently partnering with other Federal agencies to develop standard mixtures intended for this purpose, and other NIH resources may also be available to help with this.

Q12: *Given the timeframe necessary to create a consortium, will the deadlines for this RFA be extended?*
A12: At present, the NCI does not plan to extend the receipt date for applications, which is currently set at April 21, 2006. If respondents feel strongly about specific areas of the application for which this date represents a specific impairment, the NCI is willing to consider accommodating arrangements.

Q13: *How should applicants address the issues of clinical variability and the effects of chemotherapy regimens in patients whose tissues are being collected for analysis?*
A13: This project does not exclude the use of biospecimens from patients who are currently enrolled in clinical trials. In their applications, however, respondents should provide well-defined clinical protocols designed to support the overall intent of this project. This initiative seeks to develop standardized approaches to assess the capabilities of various technologies to identify proteins and peptides of relevance to cancer and use these to study molecular factors or etiologic processes associated with cancer. Respondents whose proposals utilize biospecimens from patients who are undergoing chemotherapy should be aware that agents may cause many nonspecific effects that may appear in proteomic analyses.

Q14: *What are the potential roles of industry partners in this program?*
A14: Industry and private-sector representatives may partner directly with consortia investigators, and all such partnerships or plans should be discussed in applications submitted in response to this RFA. In addition, industry representatives may interact directly with the NCI in several ongoing initiatives for large-scale production and characterization of affinity-capture reagents, and interested parties should join the NCI Clinical Proteomic Technologies Initiative for Cancer Teaming Site (https://proteomics.nci.nih.gov/teaming) to become part of a community of collaborators. All interested private-sector entities should be aware that the NCI seeks nonrestrictive licensing and commercialization so that developed tools can be made accessible to the entire research community.

Q15: *Do the costs quoted in the funding plan refer to direct costs or total anticipated costs?*
A15: The figures refer to total anticipated costs.

Q16: *What is the rationale for the decrease in annual funds awarded during the fourth and fifth years of the program, relative to those awarded during the first three years?*
A16: Costs related to sample collection and establishment of infrastructure are more pronounced in the first three years of the grant, and additional monies were allocated for these provisions.

Q17: *Does the provision that restricts funds for the purchase of capital equipment also apply to leases on instrumentation?*
A17: No. Instrumentation leases are allowed and should be included in proposals.

Q18: *How should microarray technology be incorporated with mass spectrometry (MS)-based techniques for the purposes of the RFA?*
A18: The Institute recognizes the potential of chip- and array-based approaches for analysis of clinical biospecimens and encourages applications that integrate affinity-capture- and MS-based approaches. Applicants that wish to incorporate an array-based approach should consider developing a platform based on the information gained from MS, using the candidate approach, or developing parallel MS- and chip-based platforms based on the knowledge of genes and pathways involved in cancer.

Q19: *By analyzing breakdown products in serum, one can identify proteases that are involved. Is a bioassay that uses external substrates to address these activities considered an independent assay, since the identity of the proteins remains unknown in such an approach?*
A19: [Note: The answer was deferred until more information is provided.]

Q20: *What methods are considered sufficiently high-throughput for inclusion in proposals for this project?*
A20: This RFA stipulates that respondents demonstrate the capability to perform a minimum of 10 sample runs per day.

Q21: *Are two-dimensional (2-D) gel chromatography and MS methods considered sufficiently responsive for this RFA?*
A21: No. 2-D gel techniques have limitations in throughput and reproducibility and may not be able to support analysis of proteins within the dynamic range observed in biofluids and tissue.

Q22: *Can co-principal investigators be identified in applications?*
A22: Traditionally, there is no category for "co-principal investigator" listed on an NIH application. The importance of specific investigators to an application may be demonstrated through percentages of effort and a description of their roles in the proposed program. There is precedent at the NCI, however, for applications that list one principal investigator (PI) for a limited portion of the duration of the research project, followed by another PI for the remainder of the project.

Q23: *Can consultants and external advisers be members of a CPTAC consortium's internal advisory board (IAB)?*
A23: It is highly recommended that CPTAC restrict IAB membership to investigators from the participating institution(s) who are directly involved in the science described in the application. Applications that include a series of advisers from other institutions compromise the NCI's ability to define relevant external reviewers.

Q24: *Is it required that a mouse model be included in a proposed experimental design for this application?*
A24: No. However, mouse models provide useful avenues to test protocols and procedures while conserving precious clinical samples.

Q25: *Has the NCI determined which common mouse model it will provide?*
A25: No. Determination of this common mouse model will be influenced by the collected applications and by other NCI programs that provide relevant proteomic data.

Q26: *What is the rationale for using mouse models to support this project?*
A26: In the context of this project, "mouse models" are models developed specifically for studying cancer processes. A list of NCI resources in this area is provided in the RFA. Well-characterized mouse models traditionally have been useful to minimize biological variability. Mouse models provide well-defined models of ontology and progression at the DNA and protein levels, supporting the identification of molecular signatures of cancer that will facilitate analysis of clinical specimens. Mouse models also support a candidate-based target identification strategy that is based on genes implicated in cancer processes.

Q27: *Given that some antibodies may not work across species, should applicants to this RFA focus on human or murine protein targets?*
A27: The NCI has no preference for any specific approach; however, resources developed through this RFA should contribute to studies of the clinical expression of cancer. Regardless of approach, applicants are highly encouraged to provide a rationale for their choice of strategy and the proposed development of resources. The NCI-supported reagents resources core that is part of the Clinical Proteomic Technologies Initiative for Cancer will develop antibodies to human and murine targets. By identifying epitopes identical to both species, core researchers expect to identify antibodies that have dual application for mouse and human samples.

Q28: *For the purpose of this RFA, can antibody companies use production facilities outside the United States?*
A28: Yes.

Q29: *Can companies that generate antibodies for a CPTAC application retain intellectual property (IP) rights over the reagents and sell them through their catalogs?*
A29: The NCI does not want to hamper the commercialization opportunities afforded by the partnerships within CPTAC consortia. However, the Institute also wishes to make resources widely available to the research community. Therefore, technology transfer issues should be discussed in the resource reagent sharing plan in the application. Applicants should address how developed resources can be made available to the research community in a nonrestrictive fashion, while still allowing commercialization and product development.

AFTERNOON SESSION

Q1: *How should an applicant group that has questions related to the caBIG™ compatibility of its current informatics tools develop an acceptable proposal or a system that will be caBIG™ compatible?*
A1: caBIG™ is an open, voluntary program that features specific domain areas that are organized into four workspaces: Integrative Cancer Research, Clinical Trials Management Systems, Tissue Banks and Pathology Tools, and In Vivo Imaging. Within each workspace, interest groups meet via teleconference to discuss the ongoing development of projects sponsored by that workspace. RFA applicants and other interested parties are encouraged to participate in these discussions, which can provide information regarding the status of a specific tool with regard to the caBIG™ development process. For proposals that feature proteomics, the Integrative Cancer Research Workspace is the most appropriate, and contact information for workspace leaders is available by following the Integrative Cancer Research Workspace links at http://caBIG.nci.nih.gov.

Q2: *For applicants that wish to partner with one or more companies, how much information regarding this partnership should be included in the application? What is the most appropriate format for the industry partner to express its interest in working on a project?*
A2: A clear description of the proposed partnership should be included in the body of the application. This description should provide a summary of the nature of the proposed interaction between the applicant and industry partner(s). It should be made clear to reviewers that the industry partner is willing to accept the premises of data and resource sharing that are commensurate with this application. A letter of support or evidence of a formal agreement testifying to the willingness of the industry partner(s) to participate in the project will strengthen the application, although this letter may be submitted after the deadline.

Q3: *Will scientists from industry be involved in the peer review process for this application?*
A3: Yes.

Q4: *What is the best way for applicants to address the specific requirements of the First-Generation Guidelines for NCI-Supported Biorepositories (http://biospecimens.cancer.gov/biorepositories/guidelines.asp) as requested by this RFA? In what format and in what section of the application should these guidelines be addressed?*
A4: These guidelines will be posted in the *Federal Register* in the upcoming weeks, accompanied by a 45-day public comment period. Although these guidelines are voluntary, the NCI requests that applicants read them, ask questions, and, ultimately, follow them. Applicants should email specific questions regarding the guidelines to biospecimens@mail.nih.gov. Applicants should address the guidelines, beginning with plans for sample preparation, as part of their response to Objective (2a), "Availability of Human Clinical Samples," discussed on page 8 of the RFA. In the application, this information will be included as part of the applicant's materials to support Section 2, "Scope of Research" (pp. 16-17).

Q5: *What are the provisions for data management with regard to this application, and what capabilities will caBIG™ provide to maintain and store raw data generated from funded projects?*
A5: caBIG™ allows local maintenance of data but facilitates access to those data by the larger community. Therefore, participating entities that generate data will maintain those data locally and utilize caBIG™ to allow others to access these data. At present, there are no plans for caBIG™ to supply storage space for data generated by awardees.

Q6: *With regard to the specific requirement of the collection and analysis of 200 samples, what are applicants expected to do with these samples? Should they be answering any specific questions when analyzing these samples? Is there a biomarker discovery requirement?*
A6: The intended purpose of this project is to facilitate applications of these technologies to clinical questions that will ultimately enable the discovery of biomarkers. While it is likely that candidate biomarkers will evolve from this exercise, this project is not conceived as a biomarker discovery effort *per se*. Applicants should include in their experimental designs a discussion of relevance to cancer processes. For example, experimental designs for proposed studies should include developmental timeframes representing the different stages of cancer and relevant controls from similar populations that are unaffected by disease.

Q7: *Are there guidelines in the RFA that specify the acceptable total cost related to acquiring and maintaining biospecimens?*
A7: The NCI does not place any budgetary restrictions on the prospective collection of clinical specimens, and applicants are not disallowed from designing application-specific specimen collection resources. Where possible, however, the Institute wishes to leverage from ongoing planning processes and biospecimen collections and encourages applicants to consider such approaches as avenues to maximize value to the user community while controlling the total application costs.

Q8: *The requirement for cross-site validation may expose IP from the home institution and/or its CPTAC consortium partners prior to the patent process. How will these IP issues be managed (e.g., nondisclosure agreements, materials transfer agreements)? Will the universities be responsible for these negotiations, or will the NCI facilitate these agreements? How will agreements between funded CPTAC consortia be negotiated?*
A8: Management of IP issues among members of a single CPTAC consortium is the responsibility of participating institutions. However, the NCI will be an active participant in each consortium and can assist with administrative issues by facilitating meetings and workshops to help develop common agreement types. Given the timeframe for application submission, applicants are not expected to have worked out every detail of the consortium's internal IP policies. However, applicants should acknowledge in their applications an overall rubric for IP management at the consortium level. The NCI has an internal team dedicated to crafting model agreements by facilitating negotiations and discussions among institutions. While the Institute cannot dictate that an institution adhere to a particular agreement, the Steering Committee for this project will facilitate collaborations among participating institutions. Applicants should clearly demonstrate that team members are in agreement on the principle of sharing data and resources.

Q9: *Regarding issues related to data compatibility with caBIG™ , whom can applicants contact to direct specific queries or receive guidance?*
A9: Specific queries should initially be directed to the NCI Center for Bioinformatics Help Desk (ncicb@pop.nci.nih.gov). caBIG™ compatibility questions are also addressed by the Architecture and Vocabularies and Common Data Elements (VCDE) Workspaces. The NCI will arrange to send contact information for appropriate caBIG™ representatives to all parties who have expressed interest in this RFA. All funded groups are welcome to participate in caBIG™ , and the NCI provides "mentors" from the two cross-cutting workspaces to assist funded groups with caBIG™ compatibility.

Participant Comment: It was suggested that the NCI consider extending the $5,000 limit for purchase of equipment to facilitate the purchase of additional computational power and capabilities (e.g., servers).
Dr. Downing Comment: The purpose of the capital budget restrictions was to prohibit the purchase of high-throughput MS platforms.

Q10: *Has the NCI established any parameters to monitor the prospective collection of biospecimens and to assess their quality?*
A10: The Institute has recently drafted its First-Generation Guidelines for NCI-Supported Biorepositories (http://biospecimens.cancer.gov/biorepositories/guidelines.asp), the first step toward development of standards for assessing biospecimen quality. The OBBR will continue to address these quality issues, and this project will be a key source of data and feedback regarding the development of standards. In the absence of standards, however, applicants should propose a scheme for specimen collection and provide a rationale for their choice.

Q11: *Numerous algorithms and software tools are currently available to extract MS data for proteomics experiments. What role will caBIG™ play in determining strategies for MS data mining?*
A11: caBIG™ is not a prescriptive entity; rather, it aims to provide access to as many different algorithms and techniques as possible. Although caBIG™ does have a data standards activity that ratifies best practices within its various workspaces, it will foster the interchange of numerous algorithms and mining techniques and will not selectively endorse certain algorithms or approaches in favor of other viable tools. In certain instances, such as the transfer of certain types of data sets, however, caBIG™ (in concert with CPTAC consortia) may define a standardized way to transfer data between consortia.

Q12: *Can you clarify what is meant by the term "candidate molecule" with respect to the CPTAC RFA?*
A12: A candidate molecule is one that, based on the literature or previous studies, is associated with a certain cancer. There is a reasonable expectation that the molecule will appear in biofluid such as serum or plasma. The NCI recognizes that the molecule(s) may not have been previously identified in biofluid, so applicants should propose a logical plan for searching for these molecules in a specific medium. It is expected that CPTAC consortia will likely discover new candidate molecules in the course of their experiments. The Institute wishes to avoid an empiricist approach in which unknowns and controls are sampled and proteins are identified in clinical specimens without establishing the molecules' connections to cancer biology. This RFA was developed with a biological context in mind, and the NCI has numerous cancer biology resources that can be used to inform analysis by CPTAC consortia. The Institute recognizes that some of the candidate molecules initially predicted to be present may not be measurable within the scope of available technology, but their absence may still be informative to the field.

Q13: *Does the NCI have an estimate of the number of candidate markers it expects a CPTAC consortium to study at a given time?*
A13: The Institute has not set specific targets for the number of candidate molecules to be investigated at the consortium level. Over the timespan of the program, the NCI hopes to have 1,500 proteins or peptides to be analyzed.

Dr. Downing Comment: Community input, based largely on genomic profiling, indicates a fairly strong consensus that raw data file output be captured. The NCI is currently working on ways to enable this request. Although it is not emphasized in the RFA, access to the raw MS data and analyses will be important for interlaboratory studies. The NCI recognizes that there are differences in the software output from various commercial instruments, and it has been working with instrument manufacturers to develop "translators" that will facilitate a common file output. The CPTAC also will have the capability to work with developers on the sharing of data and algorithms from the instruments that they manufacture.

Q14: *Can you clarify what is meant by the term "high throughput" with respect to this RFA?*
A14: As noted in Section IV.6.3, "Analysis Capacity of the Instrumental Resources" (p. 17), applicants should demonstrate the capacity to complete 10 MS runs/day. This benchmark was included to support those elements of this project that will be undertaken across multiple centers. The NCI wishes to avoid a scenario in which one group is limited by its MS access such that collaborating groups then become delayed. Each participating lab will be provided with samples by which to investigate variability in sample measurement across various platforms. Certain consortium activities will require that samples be processed and distributed within a reasonable timeframe, so the NCI seeks confirmation of existing capability to process a reasonable number of samples with certainty. Therefore, applicants should demonstrate that an acceptable amount of instrument time is available to support this project.

Participant Suggestion: Reviewers should be aware of the rationale for this throughput requirement when weighing the MS capacity of a given applicant. The requirement of 10 runs per day as stated in the RFA is vague and may represent several different levels of practical daily capability. Digestion and fractionation schemes influence the number of samples that can be processed within a day, and reviewers should be aware that internal consistency in the context of the overall RFA is more important than adherence to a specific number of runs per day.

Q15: *What amount of sequence coverage is expected for each identified available protein?*
A15: The NCI has not set a specific requirement or framework for sequence coverage, since coverage depends on technology platform capabilities. The Institute welcomes all innovative ideas from applicants regarding strategies to enhance sequence coverage.

Participant Suggestion: Reviewers could be made aware of the value of increased sequence coverage when evaluating proposals.
Dr. Downing Comment: This point will be considered by the NCI, even though it was not amplified in the RFA.

Q16: *Requirements for affinity methods are not detailed in the RFA. Is it expected that each consortium will compare at least two affinity methods?*
A16: The NCI does not require that applicants demonstrate the capability of multiple affinity-capture methods. The Institute recognizes that this RFA promotes an opportunity to enhance the field by developing technology to enhance mass-to-charge detection capabilities and affinity methods. Historically, MS- and affinity-based technologies have evolved from different perspectives, and the Institute welcomes opportunities to build partnerships that will enhance the capabilities of these technologies. Thus, a highly innovative application with multiple affinity approaches will be welcomed, although multiple affinity methods are not required for this project.

Q17: *Is there a minimal number of labs required for the interlaboratory reproducibility requirement?*
A17: Each sample within each application must be analyzed on a minimum of two instruments with the same type of detection system. The broader interlaboratory comparisons will be conducted though centers selected for funding. For these centers, the NCI does not stipulate with regard to the location of the laboratories involved in these comparisons.